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Is it possible to determine physiological quality and best conditions of storage of soybean seeds by isothermal calorimetry?

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ABSTRACT

Seeds of soybean, cv. DM5.8RR of two different physiological qualities were germinated and monitored by isothermal calorimetry and imbibition methods. Also, seeds stored at 25 °C under different vapor pressures were analyzed. Results showed that those seeds with lower physiological quality, lot 1 (germination power of 62%) had a much higher speed of water uptake than seeds with a germination power of 93%, lot 3. Thus, endothermic peaks could be observed in the specific thermal power time curves of germination of seeds of lot 1 which were related to anoxia. Seeds of lot 3 with different initial water content, iWC, showed three phases of imbibition where the third also involved metabolism. Apparently, when the enthalpy change value of water–seed interaction of the three phases is similar, roots protrude with vigor. Such was the case for soybean seeds with 0.084 and 0.101 g g^{-1} iWC.
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1. Introduction

Soybean seeds begin to germinate when its water content is about 50% of the seed weight [1]. It was found that the water distribution in soybean cotyledons during imbibition is similar to the protein distribution across them [2]. The two cotyledons that make up the bulk of the seed are composed mainly of proteins (ca. 40%), lipids (ca. 20%) and carbohydrates (ca. 29%, mainly cell wall polysaccharides and sugars) [2]. According with the kinetics of water uptake, hydration of seeds for germination shows three distinct phases. They are, rapid hydration (imbibition, phase 1), a lag phase (phase 2) and a steady hydration phase (phase 3 due to germination growth) [3]. A method to monitor seed germination that combines isothermal calorimetry and imbibition measurements was developed [4,5]. Calorimetric specific thermal power (p)–time (t) curves of germination were obtained at 25 °C for 10 or more individual seeds after 30 min of equilibration of the system. The p–t curves were extrapolated to $t = 0$ by relating the rate of water uptake as determined from imbibition curves with p values. This procedure can be avoided by using an isothermal titration calorimeter in which case p–t curves of germination can be recorded from the onset of imbibition. The p–t curves obtained were integrated to determine the specific metabolic enthalpies, Δh, which in turn were related to the water content of seeds at the corresponding times [4,5]. This method showed three phases of imbibition. However, these phases do not coincide with the three phases depicted by the kinetics of water uptake [3]. Phases 1 and 2 determined by the calorimetric method seem to coincide with phase 1 of the kinetics of water uptake. On the other hand, phase 3 partially coincides with phase 2 of the kinetics of water uptake. In order to establish if this method was suitable to determine physiological quality of seeds as well as the best conditions for storage with regards to water content, we have analyzed seeds of soybean, cv. DM5.8RR of three different qualities.

2. Experimental

2.1. Plant material

Seeds of soybean (*Glycine max*) cv. DM5.8RR harvested in 2010 were obtained with three different physiological quality from the Agro-Industrial Experimental Station Obispo Colombres, Tucumán Province, Argentina. We identified each batch as lot 1 with a germination power (GP) of 62%, lot 2 with a GP of 80% and lot 3 with a GP of 93%. Water content (WC) of the seeds was determined in triplicate by measuring the weight loss until it became constant at 95 °C. The resulting WC was 10.1, 6.3, and 8.4% for lots 1, 2 and 3, respectively.

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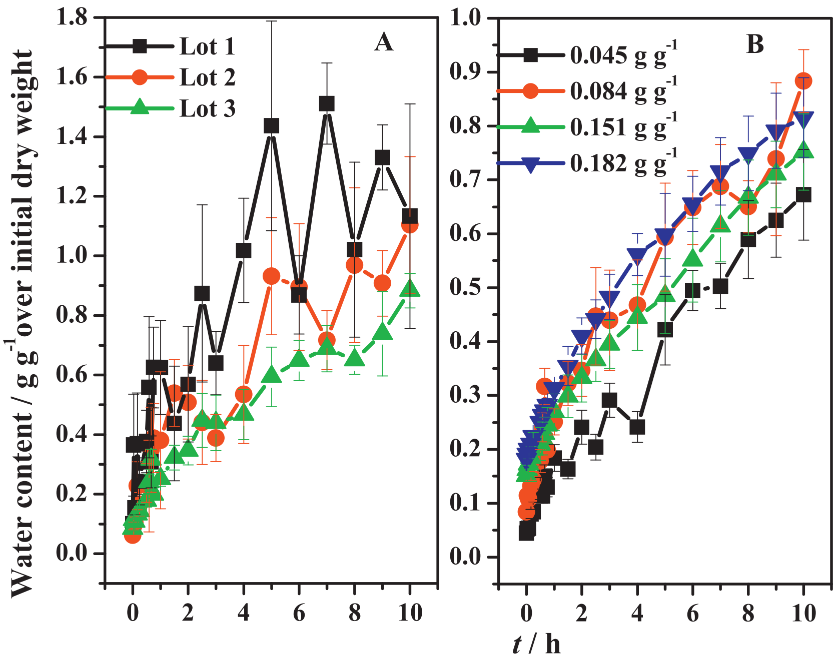

Fig. 1. Imbibition curves of soy bean seeds cv. DM5.8RR during the first 10 h (A) different physiological quality: (lot 1) lowest, (lot 2) medium and (lot 3) optimum and (B) seeds of lot 3 stored with different initial water content.

2.2. Moisture adjustment

Seeds were suspended in small aluminum foil baskets from the lids of 500 cm^3 jars over saturated salt solutions with a vapor pressure, p/p_0 ranging from 0.113 to 0.95, for 8 days at 25 °C. Moisture content was determined by drying seeds for 24 h at 95 °C. All moisture contents are expressed on a g H_2O/g dry weight basis ($g\,g^{-1}$).

2.3. Imbibition assays

Imbibition experiments were performed in a germination chamber at 25 °C by placing ten seeds in a Petri dish over a Whatman N° 1 filter paper disk with 5 cm^3 water. As measurements in the calorimeter are continuous, it was necessary that imbibition in the chamber reflects the process in the calorimeter, thus a replicate of two Petri dishes was set for each time considered. Then, at selected times, the seeds were individually weighed and returned to the dish for root emergence control. Imbibition results are reported as the mean ± SD with the WC values determined by the expression: $WC = (m_t - m_0)/m_{dw}$ where m_t and m_0 are the weight of seed at $t = t$ and $t = 0$, respectively and m_{dw} is the initial dry weight of seed. Rate of water uptake (RWU) was determined by the expression: $RWU = WC/t$ where t is the time of imbibition expressed in seconds.

2.4. Calorimetric analysis

A twin heat conduction calorimeter designed and built at Lund University, Sweden was used [4–9]. One seed was placed in the bottom of the calorimetric ampoule (8 cm^3) inserted in 1.0 ml 1% agar. As a precaution, the ampoule was opened once to allow exchange of gases after 10 to 12 h of imbibition to ensure O_2 availability. Power (P)–time (t) curves of germination were obtained after a system equilibration period of 30 min at 25 °C. The P–t curves were further converted into mass specific thermal power (p)-time (t) curves of germination by means of the seeds weight (m) expressed as dry weight basis (dw) using the equation: $p = P/m_{dw}$. A Microcal Origin program version 6.0 (Microcal Software, Inc.) was used to average values for replicate experiments and to determine specific enthalpy of imbibition and germination, $\Delta_i h$ and $\Delta_g h$, respectively from the area under each curve at the corresponding time value ($t_i = 9$ h or t_g). Results reported ($\Delta_i h$, $\Delta_g h$ and t_g) are the mean ± SD of at least ten replicates per treatment.

Values of specific thermal power were correlated with values of RWU between 0 and 90 min. From the straight line obtained and assuming that values of p between $t = 0$ and $t = 30$ min also fit in this line, the values of p were calculated for time values lower than 30 min. Then, the p–t curves were integrated to determine values of Δh at different times. These values of Δh were further correlated with values of WC at the corresponding time to determine the time and WC needed by seeds to start metabolism.

3. Results and discussion

Fig. 1A shows the imbibition curves during the first 10 h for seeds cv. DM5.8RR of different physiological quality and Fig. 1B those of seeds of lot 3 stored at different vapor pressures. Note the uneven shapes of the curves that correspond to lot 1 and lot 2 with respect to lot 3 (Fig. 1A) indicating that seeds of the former lots were not uniform in their quality. Rate of imbibition of seeds with initial water content, iWC, of 0.045 $g\,g^{-1}$ (Fig. 1B) is lower than for the other seeds with higher iWC during the first 4 h of imbibition. Then, uptake of water parallels those of seeds with higher iWC. This is not surprising because it was reported that soybean seeds with lower iWC imbibe slower than those with higher iWC [3]. The main force that drives imbibition is the difference in water potential between the seed and the surroundings. However, the speed of imbibition will depend on the permeability of the seed coat. Apparently, desiccation below a certain value causes some perturbation in the seed coat producing loss of permeability and thus, the slower water uptake [3,10].

In Fig. 2 the water sorption isotherms of soybean seeds as determined for lot 2 and lot 3 at 5 and 25 °C can be observed. The curves show a sigmoidal type pattern as previously reported [11]. Interesting is to observe that values of WC for seeds of both lots at both temperatures are not significantly different up to a p/p_0 of 0.75.

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